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Pressure Effects on the Composition and Thermal Behavior of Lipids from the Deep-Sea Thermophile *Methanococcus jannaschii*

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The deep-sea archaeon *Methanococcus jannaschii* was grown at 86°C and under 8, 250, and 500 atm (1 atm = 101.29 kPa) of hyperbaric pressure in a high-pressure, high-temperature bioreactor. The core lipid composition of cultures grown at 250 or 500 atm, as analyzed by supercritical fluid chromatography, exhibited an increased proportion of macrocyclic archaeol and corresponding reductions in archaeol and caldarchaeol compared with the 8-atm cultures. Thermal analysis of a model core-lipid system (23% archaeol, 37% macrocyclic archaeol, and 40% caldarchaeol) using differential scanning calorimetry revealed no well-defined phase transition in the temperature range of 20 to 120°C. Complementary studies of spin-labeled samples under 10 and 500 atm in a special high-pressure, high-temperature electron paramagnetic resonance spectroscopy cell supported the differential scanning calorimetry phase transition data and established that pressure has a lipid-ordering effect over the full range of *M. jannaschii*'s growth temperatures. Specifically, pressure shifted the temperature dependence of lipid fluidity by ca. 10°C/500 atm.

For archaea to flourish in the extreme environments that many inhabit, normal membrane structure and function must be preserved. Preservation of membrane function may derive partly from the unique physical structures of archaeal lipids, which differ greatly from those of their eubacterial counterparts (7, 16–18). In contrast to the straight or minimally branched, mostly unsaturated fatty acids of variable lengths found in eubacteria, archaeal apolar chains are based on saturated isopranoid alcohols typically containing 20 or 40 carbon atoms. These isopranyl chains are connected to a glycerol (or more rarely, a complex polyol) head group by ether bonds, not the fatty acid ester bonds common to eubacteria. Moreover, the stereochemistry of these ether lipids (D or *sn*-2,3) is opposite to that seen in eubacteria (L or *sn*-1,2). Some archaea also possess bilayer-spanning tetraether lipids, in which a pair of biphytanyl-based chains are linked to two glycerol moieties via ether bonds. In total, the structural differences are pronounced enough to raise the question of whether archaeal lipids respond differently to temperature and pressure than do eubacterial lipids, thus contributing to microbial viability under extreme conditions.

Given that eubacterial thermophiles and barophiles do exist (6), the unique structural features of archaeal lipids are not necessarily required for retention of membrane structure and function at elevated temperatures and pressures. However, considering the abundance of archaea and relative scarcity of eubacteria at the outer regions of the pressure-temperature envelope at which life is known to exist, many archaea appear to possess an intrinsic capacity for thermophilic and barophilic behavior.

To adapt to moderate fluctuations in temperature, virtually all eubacteria can maintain optimal membrane fluidity by modulating their lipid composition in a process termed homeoviscous adaptation (26). Even for thermophilic eubacteria, this is primarily achieved by altering the degree of saturation, branching, and chain length of the core fatty acid chains (18). Several archaea respond similarly to variations in growth temperature, although with changes limited to shifts in the distribution of core lipids produced by that microorganism (7, 27). Nevertheless, this behavior suggests that archaea, in a fashion similar to that of eubacteria, utilize the different physical properties of various core lipids to help maintain membrane structure and function at elevated temperatures.

In the deep ocean and assorted geothermal formations, microorganisms can experience significant environmental variations in pressure and temperature. In general, increased pressure affects membrane behavior in a manner similar to decreased temperature (20); thus, alterations in lipid composition at elevated pressures may be necessary to maintain optimal membrane fluidity. Such composition shifts have been previously observed in several barophilic psychrophiles (6, 29). In essence, the response may be analogous to homeoviscous adaptation, although it stems from variations in pressure rather than temperature.

Methanococcus jannaschii is a thermophilic methanogen originally isolated from a deep-sea hydrothermal vent at a depth of ca. 2,600 m (260 atm [1 atm = 101.29 kPa]). The optimum growth temperature of *M. jannaschii* at atmospheric pressure is 85°C (13). Previously, both methanogenesis and growth of *M. jannaschii* at 86 and 90°C were observed to accelerate with increased hyperbaric pressure of as much as 750 atm (23). Moreover, the upper temperature limit for methanogenesis was extended from 94°C at 10 atm to 98°C at 250 atm. Structurally, in addition to the archaeol (2,3-di-*O*-phytanyl-*sn*-glycerol) and caldarchaeol (2,2′,3,3′-dibiphytanyldiglycerol) lipids typically found in methanogens, *M. jannaschii* possesses a macrocyclic archaeol (2,3-di-*O*-cyclic-biphytanyl-*sn*-glycerol) core lipid that has so far been found only in *M. jannaschii* (5, 8) and another strain of thermophilic methanococci isolated from hydrothermal vent sediment (14). This raises the question of whether the unusual membrane lipids of *M. jannaschii* contribute to its ability to thrive at extremely high temperatures and pressures, at which it exhibits both thermophilic and barophilic behavior.

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In this work, the effect of pressure on the core lipid composition of *M. jannaschii* was investigated by performing batch fermentations in a high-temperature, high-pressure reactor system with subsequent lipid analysis by supercritical fluid chromatography (SFC). Continuous cultivation studies were also carried out to assess the effect of growth rate on core lipid composition. Previously, increasing the growth temperature of *M. jannaschii* from 47 to 75°C was observed to shift the core lipid composition from predominantly archaeol to mostly macrocyclic archaeol and caldarchaeol (27). The present experiments addressed whether pressure induces a comparable or contrasting shift.

In addition, the effect of temperature and pressure on the membrane fluidity of a model system of core lipids from *M. jannaschii* was examined by high-pressure electron paramagnetic resonance (EPR) spectroscopy. In eubacterial lipids, increased pressure exerts a solidifying effect of about 15 to 25°C/1,000 atm. The lipid-ordering effect is reflected in the increased temperature for the main liquid crystalline-to-gel (LC→G) phase transition and by reduced membrane fluidity in the liquid crystalline phase, as measured by various order parameters (20). Our experiments explored whether the model core lipid system from *M. jannaschii* exhibits similar behavior or possesses special properties that might contribute toward the thermophilicity and barophilicity displayed by the microorganism.

MATERIALS AND METHODS

High-pressure, high-temperature reactor system. For studies at elevated hyperbaric pressure, *M. jannaschii* was grown in a high-pressure, high-temperature reactor system described elsewhere (21), with the following modifications. To minimize exposure of the culture to potentially reactive metal surfaces, a removable glass liner was fitted inside the stainless steel vessel (25). A hand-operated hydraulic pressure generator was installed after the pneumatic compressor to achieve more rapid and precise hyperbaric pressurization.

Continuous culture system. For the continuous cultivation studies and the production of lipids for EPR studies, *M. jannaschii* was grown in a continuous culture system described elsewhere (28), with the following modifications. To minimize exposure of the culture to potentially reactive metal surfaces and to facilitate the use of smaller working volumes, the 5-liter fermentor was replaced with a 2-liter side-armed glass culture flask mounted on a magnetic stir plate and equipped with a Teflon stir bar. Glass tubing was used for all internal inlet and outlet lines. To maximize gas-liquid contact, gaseous substrate (a 4:1 mixture of H_2 and CO_2) was fed to the liquid phase by using a glass tube with a fritted tip.

Growth of *M. jannaschii*. Stock cultures of *M. jannaschii* were obtained from the Deutsche Sammlung Mikroorganismen (Braunschweig, Germany; culture no. 2661). Inocula of *M. jannaschii* were prepared on defined growth medium in Hungate bottles by strict anaerobic techniques as described by Balch and Wolfe (2). The growth medium contained the following components (in grams per liter): K_2HPO_4, 0.14; $CaCl_2 \cdot 2H_2O$, 0.14; NH_4Cl, 0.25; $MgSO_4 \cdot 7H_2O$, 3.4; $MgCl_2 \cdot 2H_2O$, 2.7; KCl, 0.33; NaCl, 30; and PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], 1.51. The medium also contained 1 ml of 1 mM SeO_2 and 10 ml of a trace mineral solution per liter (31). The pH was adjusted to 6.8 with NaOH before the addition of 0.5 g of $Na_2S_2O_3$, 10 ml of titanium (III) reductant (24), and 0.4 ml of β-mercaptoethanol per liter. The gaseous headspace in the inoculum bottles was purged with substrate gas and then pressurized to 20 psi. The inoculum bottles were incubated in a shaker bath at 80°C until the culture reached late exponential phase, typically in 6 to 8 h.

To initiate a high-pressure batch culture, 65 ml of freshly inoculated growth medium (~1% [vol/vol]) was transferred to the high-pressure reactor which was preheated to 86°C. The reactor was then pressurized to 8 atm with substrate gas. For growth at 250 and 500 atm, additional pressurization was achieved with He. Gas analysis for H_2, CH_4, and CO_2 was performed with an HP 5890A gas chromatograph via a sampling line connected to the reactor. To maximize production of cell mass, the reactor was periodically replenished with fresh substrate gas after methane production surpassed 50%. To this end, the reactor headspace was serially purged and repressurized with He to remove CH_4 while keeping pressure decreases below 20%. Substrate gas was subsequently added to maintain a partial pressure of 8 atm. To minimize cell lysis at harvest, the fermentation broth was collected only after cooling and gradual depressurization to atmospheric pressure.

To initiate continuous cultivation, 50 ml of inoculum was transferred into the anaerobic continuous culture system containing 1.5 liters of medium preheated to 86°C. Gaseous substrate was bubbled through the medium at a calibrated gas flow rate of 9 liters/h. Continuous operation was initiated during late exponential phase. Gas analysis for CH_4 and CO_2 was performed with the HP 5890A gas chromatograph. Bacterial growth was monitored by measuring the A_{600} in a Beckman DU-6 spectrophotometer. Fermentation broth was collected after at least 4 reactor volumes to allow the continuous system to reach a steady state. Cell mass was harvested from the cooled broth by centrifugation and frozen for later use.

Isolation of lipids from *M. jannaschii*. Total lipid extracts were prepared by modifying the procedure described by Bligh and Dyer (3). For *M. jannaschii* from continuous culture, 0.50 g of thawed cell mass was suspended in 27 ml of water with a hand homogenizer. A trace amount of DNase was added to minimize DNA interference with later extraction steps. The cell suspension was combined with 90 ml of 2:1 CH_3OH-$CHCl_3$ and stirred for at least 2 h. After addition of 30 more ml of $CHCl_3$ and 27 ml of water to the extraction mixture, the phases were allowed to separate overnight. To recover the total lipid extract, the lower $CHCl_3$ phase was collected and evaporated to dryness. For *M. jannaschii* from the high-pressure reactor, the cells in growth medium were concentrated in an Amicon ultrafiltration cell using an Amicon Diaflo YM1 membrane (1,000-molecular-weight cutoff). The concentrated cell broth was diluted to 27 ml with water and was used directly in the extraction procedure described above.

To isolate the neutral lipid extract, the total lipid extract was twice suspended in 5 ml of ice-cold acetone in which the polar lipids were insoluble. The acetone washes were carefully collected, pooled, and dried under a stream of filtered nitrogen. The polar lipid extract was then recovered by dissolving the remaining solid residue in $CHCl_3$. Although small amounts of archaeol and macrocyclic archaeol were detected in the neutral lipid extract, control experiments showed no significant difference in the core lipid composition of lipid extracts from continuous culture prepared with and without acetone precipitation.

Core lipids were prepared from the polar lipid extract by strong acid hydrolysis (12). The polar lipid fraction was dissolved in 250 μl of $CHCl_3$ and added to 2 ml of 10:1 CH_3OH-HCl in a glass vial with a Teflon-lined screw cap. The vials were incubated at 95°C in a water bath for 1 h. After cooling, 4 ml of water was added to the hydrolysate, which was subsequently extracted three times with 4 ml of 4:1 hexanes-$CHCl_3$. The hexanes-$CHCl_3$ fractions were pooled and dried under a stream of filtered nitrogen.

Core lipid analysis by SFC. Analysis of core lipid composition (12) was performed on a Lee Scientific Series 600 SFC/GC (Lee Scientific, Salt Lake City, Utah). The column was an SB-Biphenyl-30 (Lee Scientific) (10 m; outside diameter, 195 μm; inside diameter, 50 μm) with a 0.25-μm film thickness. The mobile phase was SFC grade CO_2 (Matheson Specialty Gases, Fremont, Calif.) supplied from a cylinder equipped with a dip tube and no helium headspace.

All SFC analyses were performed isothermally at an oven temperature of 120°C. The pressure profile for a typical run began at 40 atm, rose to 233 atm at 3.3 atm/min, and then increased at 6.6 atm/min to a final pressure of 367 atm. The flame ionization detector was set at 350°C and supplied with 30 ml of H_2, 35 ml of N_2, and 300 ml of air per min. Peaks were assigned by analyzing purified core lipid standards previously isolated by thin-layer chromatography, the structures of which were verified by high-resolution mass spectrometry.

Preparation of spin-labeled lipid dispersions. Dipalmitoyl-L-α-phosphatidylcholine (DPPC) and *n*-octyl-β-D-glucopyranoside (*n*-octyl glucoside) were purchased from Sigma. The spin label 5-DOXYL stearic acid was purchased from Aldrich.

For spin-labeled lipid dispersions prepared by simple agitation (15), a measured amount of lipid was dissolved in $CHCl_3$ and transferred to a glass vial. A solution of 5-DOXYL stearic acid dissolved in $CHCl_3$ was added to achieve a spin label concentration of ~1 mol% (4). The sample was dried under filtered N_2 and stored under house vacuum (~10 mm Hg) for several hours to remove any residual $CHCl_3$. Buffer (50 mM 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid [EPPS] [pH 7.4]) was added to achieve a lipid concentration of 15 to 30 mg/ml. The sample was swirled at an elevated temperature for 5 to 15 min and then sonicated for 60 min in a Bransonic 12 sonication bath.

For spin-labeled lipid dispersions prepared by detergent dialysis (19), the lipids were labeled as described above. In a typical preparation, 2.5 mg of spin-labeled lipid was solubilized in 0.6 ml of 50 mg of *n*-octyl glucoside stock solution per ml. The detergent mixture was swirled briefly and then sonicated for 30 min at an elevated temperature. The clear solution was dialyzed against 250 ml of 50 mM EPPS (pH 7.4) buffer for at least 12 h and redialyzed twice more against fresh buffer. After dialysis, the slightly turbid solution was concentrated in an Amicon Microcon-3 microconcentrator (3,000 MWCO) spun at 10,000 rpm in a microcentrifuge.

DSC studies. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer 1020 DSC 7 thermal analysis system. For each sample, 50 μl of spin-labeled lipid dispersion was placed in an sealed stainless steel pan (60-μl capacity) capable of withstanding 10 atm of pressure. As a reference standard, 50 μl of 50 mM EPPS (pH 7.4) buffer was used. Scanning rates were either 2 or 5°C/min. Multiple thermograms (generally 5 to 10) were recorded for each sample until no differences between scans were visible. Control experiments revealed no differences between the thermal behaviors of samples with and without spin label.

EPR spectroscopy studies. Spectra were recorded on a Brüker ER200D-SRC EPR spectrometer with a microwave power of 12.6 mW, modulation amplitude of 1.0 G (1 G = 0.1 mT), and scan range of 100 G. Sample temperature was controlled with an ER4111-VT variable temperature unit with the purge gas flow

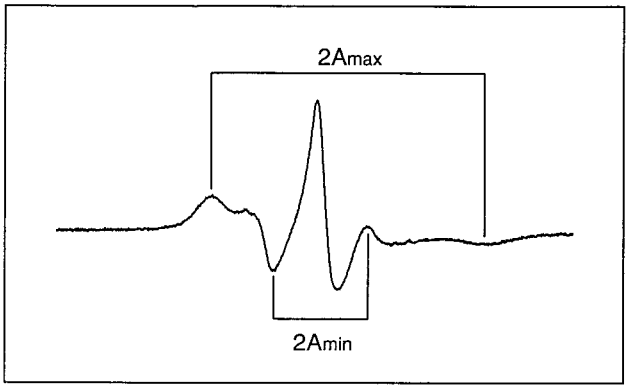

FIG. 1. Schematic of the order parameter calculation from the EPR spectra of a sample 5-DOXYL stearate-labeled lipid dispersion (10), in which the order parameters S is defined as follows:

$$S = \frac{A_{max} - \overline{A}_{\perp}}{A^c_{zz} - \frac{1}{2}(A^c_{xx} + A^c_{yy})} k, \text{ where } k = \frac{A_{max} - 2\overline{A}_{\perp}}{A^c_{xx} + A^c_{yy} + A^c_{zz}}$$

$$\text{and } \overline{A}_{\perp} = A_{min} + 1.32 + 1.86 \log \left\{ 1 - \frac{A_{max} - A_{min}}{A^c_{zz} - \frac{1}{2}(A^c_{xx} + A^c_{yy})} \right\}$$

rate set to 5 liters/h. For measurements at atmospheric pressure, 25 μl of lipid dispersion was transferred via a syringe and intramedic tubing to a glass capillary tube. The open end of the tube was then flame sealed to minimize liquid loss at elevated temperatures.

For measurements at elevated hydrostatic pressures, a high-pressure EPR cell described elsewhere (1) was used, with the following modifications. The lipid dispersion was transferred via a syringe to ca. 2 cm of intramedic tubing (1.19 by 1.70 mm [inside and outside diameter, respectively]). The tube was then heat sealed at both ends and placed in the high-pressure cell filled with low-dielectric Dow Corning 200 silicon oil as a pressurizing fluid. The o-ring–high-pressure fitting was carefully mated to the top of the cell and screwed into place. After positioning of the high-pressure cell in the detection window of the EPR cavity, the high-pressure fitting was connected to the manual piston screw pump (HIP pressure generator), which had been previously flushed and filled with distilled water. In practice, the sample is able to withstand repeated pressurization and depressurization at temperatures of as much as 100°C.

Calculations of the order parameter S (see legend to Fig. 1 for definition) were made by the method described by Griffith and Jost (10). The spectral parameters $2A_{max}$ and $2A_{min}$ were measured directly from the digitized EPR spectra. Literature values for A^c_{xx}, A^c_{yy}, and A^c_{zz} of 5-DOXYL stearate were used, along with k, the polarity correction to the reported single crystal values. A diagram of the spectral measurements and equations used for the calculations are shown in Fig. 1 and its legend, respectively. Theoretically, for these systems, decreases in S correspond to increases in membrane fluidity and vice versa.

RESULTS

Pressure effects on core lipid composition. To examine the effect of pressure on the core lipid composition of *M. jannaschii*, cells were grown at 86°C under 10, 250, and 500 atm of hyperbaric pressure in the high-temperature, high-pressure reactor system. Consistent with *M. jannaschii*'s barophilicity, methanogenesis proceeded faster at the higher pressures, with the specific growth rates calculated from methanogenesis being slightly greater than those previously reported (23). The core polar lipid composition data are summarized in Table 1, and a representative SFC chromatogram is shown in Fig. 2. As shown by Sprott et al. (27), polar lipids constitute as much as 92% of the total lipid content of *M. jannaschii*. Compared with the 10-atm cultures, the cells grown at 250 and 500 atm exhibited a marked shift toward macrocyclic archaeol with reductions in both archaeol and caldarchaeol (the former being undetectable at the higher pressures). No differences were observed between the 250- and 500-atm data.

Because the high-pressure data were determined from batch fermentations with variable endpoints, the results could be skewed if the core lipid composition shifts significantly with growth rate. To address this concern, *M. jannaschii* was grown in continuous culture at dilution rates ranging from 0.13 to 0.33 h^{-1}, the maximum dilution rate achievable in the current system. On the basis of subsequent analysis by SFC, varying the growth rate over this range did not affect the core lipid composition (data not shown).

TABLE 1. Distribution of core polar lipids in *M. jannaschii* under various growth conditions

Growth condition	Composition (%)		
	Archaeol	Macrocyclic archaeol	Caldarchaeol
1 atm, 75°C[a]	18	36	46
1 atm, 86°C[b]	7 ± 3	52 ± 5	41 ± 4
250 atm, 86°C[b]	0–2	65 ± 5	35 ± 5
500 atm, 86°C[b]	0–2	64 ± 6	36 ± 6

[a] Numbers estimated from data described by Sprott et al. (27).
[b] Grown in the high-pressure temperature reactor. Values are the means of duplicate measurements from two separate fermentations. The values for archaeol at 250 and 500 atm span the measured value (zero) and the estimated amount that may have been lost in the neutral lipid extract. All other errors are mean deviations of the duplicate measurements.

DSC of DPPC and *M. jannaschii* lipids. To study the thermotropic behavior of *M. jannaschii* core lipids at atmospheric pressure, DSC was performed with DPPC as a control. For this and all other EPR studies, the model *M. jannaschii* core lipid system was obtained exclusively from cell mass grown in continuous culture at a dilution rate of 0.23 h^{-1} and was composed of 23% archaeol, 37% macrocyclic archaeol, and 40% caldarchaeol (as analyzed by SFC). Representative thermal scans for DPPC and *M. jannaschii* are shown in Fig. 3. As expected, the thermogram for DPPC exhibited a distinct peak corresponding to an endothermic phase transition at 41 to 42°C. In contrast, the thermogram for the model *M. jannaschii* core lipid system displayed no well-defined phase transition over the temperature range of 20 to 120°C, at least within the sensitivity limits and signal-to-noise level of the instrument.

Pressure effects on thermotropic behavior of DPPC. To investigate the effect of temperature and pressure on the membrane fluidity of core lipids from *M. jannaschii*, a special high-pressure EPR technique was employed. To test the accuracy of the EPR technique, 5-DOXYL stearate-labeled DPPC was analyzed as a control.

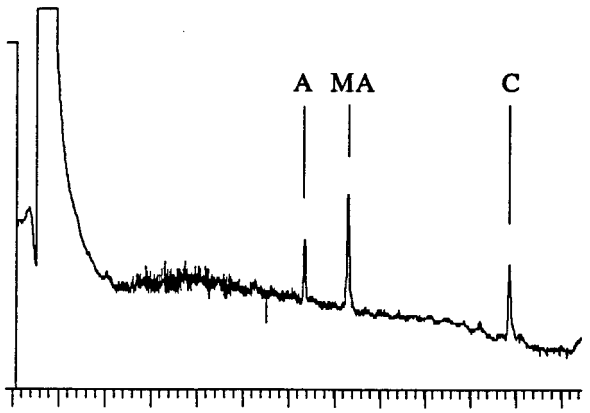

FIG. 2. Sample SFC chromatogram of *M. jannaschii* core lipids. Peak identities are as follows: A, archaeol; MA, macrocyclic archaeol; C, caldarchaeol.

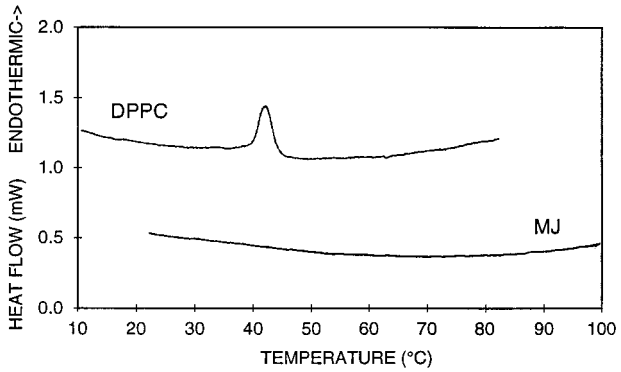

FIG. 3. DSC measurements in excess water of DPPC and a model core lipid system from *M. jannaschii* (MJ) (23% archaeol, 37% macrocyclic archaeol, and 40% caldarchaeol).

EPR spectra were recorded for DPPC from 30 to 80°C at pressures of 10, 250, and 500 atm, and the order parameter S was calculated via spectral analysis. The effect of temperature and pressure on S is plotted in Fig. 4. As evidenced by the abrupt decrease in S, or increase in membrane fluidity, the temperature of the main LC→G phase transition at 10 atm was roughly 41 to 42°C, which correlates well with the DSC data. At 250 atm, the transition was shifted to 45 to 46°C, while at 500 atm the transition was close to 51 to 52°C. On average, the observed increase in transition temperature with increasing pressure was approximately 0.02°C/atm. In addition, the temperature dependence of membrane fluidity on either side of the phase transition was similar at all three pressures. In the liquid crystalline phase, the fluidity-temperature curves exhibited comparable slopes but were shifted to elevated temperatures.

Pressure effects on thermotropic behavior of *M. jannaschii* core lipids. EPR spectra were also recorded for the model *M. jannaschii* core lipid system from 35 to 100°C at pressures of 10 and 500 atm. The effect of temperature and pressure on S is plotted in Fig. 5. In contrast to DPPC, but consistent with the DSC data, the model *M. jannaschii* core lipid system exhibited no LC→G phase transition at either 10 or 500 atm in the temperature range studied. Instead, the membrane fluidity increased steadily from 35 to 100°C, which spans the temperature range of growth for *M. jannaschii*. However, the fluidizing

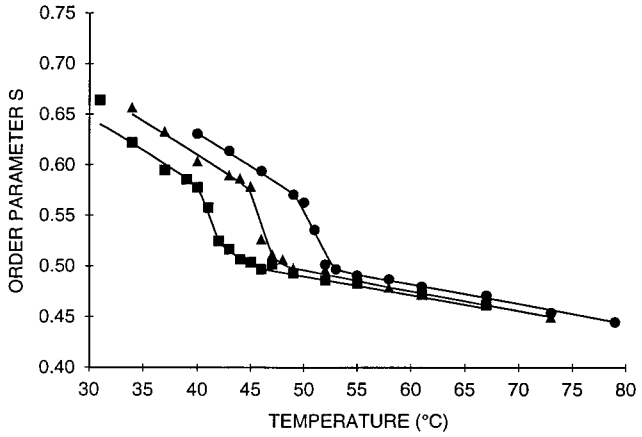

FIG. 4. Effect of pressure on the thermotropic behavior of DPPC as determined by EPR measurements of 5-DOXYL stearate-labeled lipid dispersions (10 [■], 250 [▲], and 500 [●] atm).

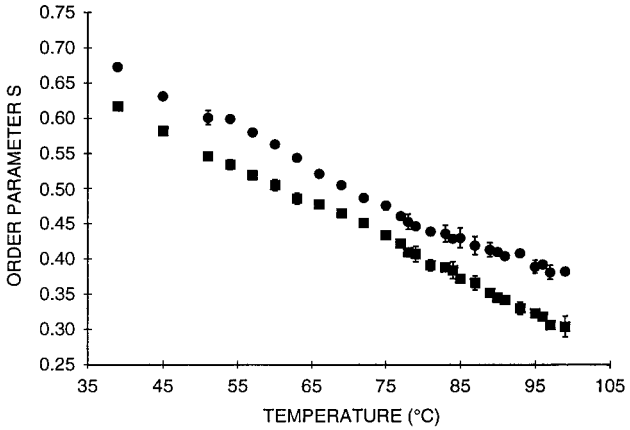

FIG. 5. Effect of pressure on the thermotropic behavior of a model core lipid system from *M. jannaschii* (23% archaeol, 37% macrocyclic archaeol, and 40% caldarchaeol) as determined by EPR measurements of 5-DOXYL stearate-labeled lipid dispersions (10 [■] and 500 [●] atm).

effect of temperature was offset by the lipid-ordering effect of pressure (by about 10°C/500 atm). Thus, pressure has a pronounced effect on the physical state of the model membrane.

DISCUSSION

In a previous study (27), Sprott et al. found that increasing the growth temperature of *M. jannaschii* induced a shift in core lipid composition from predominantly archaeol to mostly macrocyclic archaeol and caldarchaeol. Within the context of homeoviscous adaptation, the general explanation for this behavior is that the microorganism shifts to more rigid core lipids to counteract increased membrane fluidity at the higher temperatures. For macrocyclic archaeol, a reduction in motion due to cyclization of the core phytanyl chains is thought to impart greater rigidity to the membrane. For caldarchaeol, spanning the membrane and effectively binding two head groups is believed to reduce lateral diffusion on both sides of the bilayer.

In this work, increasing the growth pressure of *M. jannaschii* induced a much different response as the core composition shifted toward macrocyclic archaeol from archaeol and caldarchaeol. Although the decrease in caldarchaeol is understandable in terms of increasing membrane fluidity against the ordering effects of pressure, the apparent loss of archaeol at the higher pressures is puzzling. These results are in contrast to limited data for barophilic psychrophiles, in which increased growth pressure shifted core lipid composition in a manner similar to that of decreased growth temperature (6, 29). It is thus possible that the macrocyclic archaeol, which is predominant at the higher pressures in *M. jannaschii*, serves some special role at elevated temperatures and pressures.

As evident in the DSC results, no LC→G phase transition is observed for the model *M. jannaschii* core lipid system from 20 to 120°C. Although relatively small phase transitions have been identified for a variety of purified archaeols and caldarchaeols, because their thermal behavior is dominated by the core isopranyl chains it is likely that most archaeal lipids are in the fluid state above 0°C and certainly at growth temperatures (16). The absence of a major phase transition over the growth temperature range could prove advantageous, for one theory speculates that microbial viability in extreme environments may be enhanced by membrane stability over broad temperature and pressure ranges (18). For example, compared with a model diether or DPPC, a chemically synthesized model of

caldarchaeol more effectively retained low- and high-molecular-weight compounds over a considerable temperature span (30).

The EPR data for the model *M. jannaschii* core lipid system indicate that while increased temperature increased membrane fluidity, the application of high pressure reduced membrane fluidity for all temperatures tested. This behavior is entirely consistent with the observed behavior for eubacterial lipids (20). Increased temperature tends to raise membrane fluidity due to the increased thermal energy of the system. In comparison, an increase in pressure exerts an ordering effect that favors the more compact lamellar gel phase over the liquid crystalline phase and thus tends to lower membrane fluidity. However, consistent with the DSC data and in contrast to the DPPC control, no phase transitions were evident in the EPR data at temperatures of as much as 100°C.

Efforts to explain the effect of pressure on complex metabolic processes such as methanogenesis in *M. jannaschii* must consider many interrelated factors. First, the key enzymatic pathway(s) must be thermostable and barostable to the specific conditions of interest. Furthermore, membrane-mediated processes crucial to the pathway must exhibit similar thermostability and barostability. In a broader sense, a microorganism that survives and fluorishes at elevated temperatures and pressures must not possess a crippling rate-limiting pathway. For *M. jannaschii*, the upper temperature limit for methanogenesis was observed to increase from 94°C at 10 atm to 98°C at 250 atm (23). Hydrogenase, one of the key enzymes in the methanogenic pathway, has already been shown to be both more active and stable at high temperatures and pressures (11, 22). From the EPR data on the model core lipid system, this upward shift of 4°C in the upper temperature limit over a span of 240 atm is consistent with and may be related to the observed shift in the membrane fluidity behavior, which is of similar magnitude.

In previous studies with the Na^+/K^+ ATPase from marine teleost fishes (9), it was shown that changes in membrane fluidity via altered lipid composition had a direct effect on membrane-associated enzyme function. Similar changes in membrane fluidity could easily arise from shifts in environmental temperature or pressure.

In summary, pressure stabilization (or activation) of a microorganism may derive from a variety of sources, including pressure-enhanced enzyme stability (11) and shifts in lipid composition and/or intrinsic membrane properties with respect to temperature and pressure. Although a great deal of research on various enzyme systems under extreme conditions has been performed, much remains to be understood about the contributions of membrane lipid composition and of membrane physical properties to the observed thermostability and barostability of many archaea in their native environments.

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